Joint action of natural and synthetic photosystem II inhibitors

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Abstract: The inhibitory action on photosystem II of four sorgoleone analogues, isolated from *Sorghum bicolor* (L) Moench, and two synthetic inhibitors, diuron and bentazone, was tested by measuring oxygen evolution of thylakoid membranes. The inhibition of oxygen evolution for mixtures of inhibitors was compared with the Additive Dose Model (ADM). ADM assumes that, at a defined response level, the effect of a mixture of inhibitors can be unambiguously expressed by the potency of either of the inhibitors applied separately. The slope of the logistic dose-response curves differed between the inhibitors; sorgoleone analogues had the steepest and bentazone the shallowest slope. The difference in slopes makes the interpretation of the isoboles less general and may reflect the differences in the interaction between the natural and the synthetic inhibitors with the binding site. The results suggest that there may be some limitation to ADM, namely that compounds with the same site of action might have different response curves if their mechanism of binding is different. The joint action of inhibitors follows ADM at I_{50}. Therefore, the inhibitors can replace each other in any mixture ratio, based on the relative potencies of the pure inhibitors, without changing each other's effect on oxygen evolution. The joint action at I_{20} and I_{80} sometimes diverged from ADM.
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Keywords: mixtures; oxygen evolution; sorgoleone; diuron; bentazone; isobole; Additive Dose Model; dose-response curves

1 INTRODUCTION

Sorgoleone is the major component of a group of structurally related phytotoxic benzoquinones excreted by the roots of *Sorghum bicolor* (L) Moench.[1] The mode of action of sorgoleone involves the inhibition of electron transfer between Q_A and Q_B at the reducing side of photosystem II (PSII).[2–4]

Einhellig and Souza[2] found some differences in sensitivity to sorgoleone among species tested. They measured total biomass of seedlings grown hydroponically. On the basis of their data, the concentration required to reduce growth by 50% relative to the growth without sorgoleone, ie I_{50}, ranged from *c* 10–60 μM for three dicotyledenous plants to between 1 and 30 μM for three grasses. Nimbal and Weston[5] found, also in hydroponically grown plants, I_{50} values ranging from 10 to more than 200 μM by measuring biomass of some common weeds: *Echinochloa crus-galli* (L) Beauv, *Abutilon theophrasti* (L) Medic, *Ipomea hederacea* (L) Jacq, and *Digitaria sanguinalis* (L) Scop, the last being the most sensitive. By using a scale of symptoms, Rimando *et al*[6] found that 3 mM of four sorgoleone analogues, of which sorgoleone and 5-ethoxysorgoleone are natural analogues, and 2,5 dimethoxysorgoleone and hydrogenated sorgoleone are synthetic modifications, severely damaged but did not kill lettuce seedlings, whereas atrazine in this test reduced seedling growth at 0.1 mM.

Sorgoleone is a potent inhibitor of photosynthetic O_2 evolution from isolated thylakoid membranes with an I_{50} of 0.1 μM for spinach (*Spinacia oleracea* L) which was similar in value to that of the commercial herbicide diuron.[3] The I_{50} value found in isolated pea (*Pisum sativum* L) thylakoids was twice as high, but this potency was not compared with any synthetic PSII inhibitor.[7] Rimando *et al*[6] measured oxygen evolution and found I_{50} values of 0.1, 0.28, 1.07, and 0.93 μM for sorgoleone, 2,5-dimethoxysorgoleone, 5-ethoxysorgoleone and atrazine, respectively.

Sorgoleone has the same binding site as the commonly used synthetic PSII inhibitors, namely at the same Q_B niche on the D_1 protein. Sorgoleone is a strong competitive inhibitor that has been shown to displace atrazine from the Q_B binding site.[3,4]

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Nimbal *et al*[3] also showed that bentazone did not displace atrazine, and hypothesized that it might be a poor competitor with a binding site different from that of atrazine and consequently from sorgoleone.

Because of the putative similar actions of sorgoleone analogues and other PSII inhibitors, the Additive Dose Model (ADM) could be used as reference for assessing the joint action of their mixtures.[8–10] The ADM assumes that, at a defined response level, the effect of a mixture of two inhibitors can be expressed unambiguously by the potency of either of the two inhibitors applied separately. If we wish to analyse the joint action at I_{50} which is achieved with 4 μM of inhibitor A and 1 μM of inhibitor B singly, then the two inhibitors can be mixed in any proportion without changing the response level of I_{50} by using their relative potency of 4 (4/1). For example, if we want to make a mixture with 2 μM of inhibitor A then we must add 0.50 μM (2/4) of inhibitor B to obtain a response level of 50%. ADM is analogous to the exchange of currencies, the exchange rate between currencies resembling that of relative potency between inhibitors.[11] To our knowledge there are no reports of the application of ADM to assess the joint action of PSII inhibitors at the molecular level.

The objective of this paper was to test the hypothesis that a joint action model, assuming similar site of action and competitiveness at the target site (ADM), is appropriate to describe the action of mixtures of sorgoleone analogues and synthetic PSII inhibitors at the molecular level.

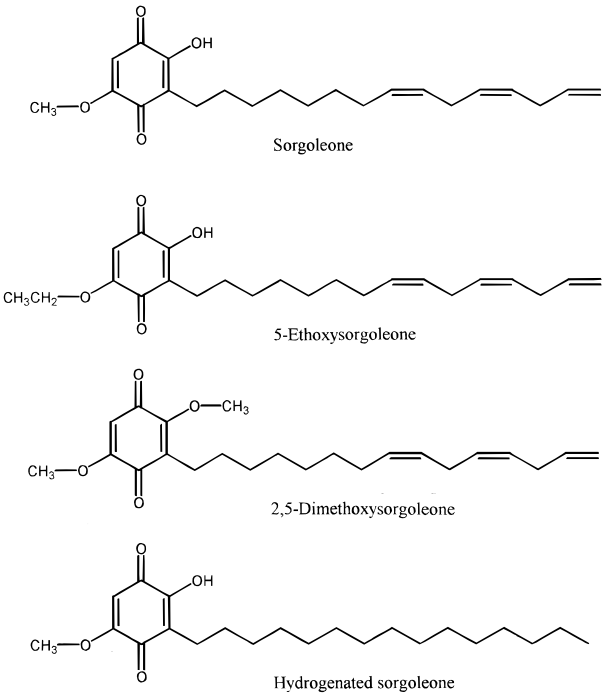


Figure 1. Structure of sorgoleone analogues. Sorgoleone and 5-ethoxysorgoleone are naturally occurring in *Sorghum bicolor*, 2,5-dimethoxysorgoleone and hydrogenated sorgoleone are synthetic modifications.

2 MATERIALS AND METHODS

2.1 Extraction and purification of sorgoleone

Sorgoleone was extracted from sorghum roots using acidified chloroform as described by Rimando *et al*.[6] Purified sorgoleone was either used in its native form or methylated, ethoxylated or hydrogenated as described elsewhere[6] (Fig 1).

2.2 Isolation of thylakoid membranes

Photosynthetically active thylakoid membranes from spinach (*Spinacia oleracea* L.) were isolated using methods described elsewhere.[3,6] Chlorophyll concentration was adjusted to 4 mg ml^{-1} before storing the membrane preparations at −80°C.

2.3 Dose-response assays

All assays were performed at 30°C. Test compounds were dissolved and diluted in ethanol. All treatments including the untreated control received the same amount of ethanol (<10 ml litre^{-1}). The reaction assay buffer consisted of sucrose (800 mM), Mes-NaOH (50 mM; pH 6.2), $CaCl_2$ (15 mM), FeCN (1 mM). Thylakoid membranes were incubated with inhibitor on ice for at least 30 min prior to measurement. The thylakoid membranes (50 μl) were mixed with 2950 μl of reaction assay buffer and equilibrated for 10 s. Oxygen evolution was measured for 20 s under saturating light conditions (2.4 mmol m^{-2} s^{-1} photosynthetically active radiation) using a fibre optic light source, and O_2 concentration was measured polarographically using a computer-controlled Hansatech DW1 oxygen probe. The slope of the linear relationship between O_2 evolution and time was calculated.

The range of inhibitor concentrations used was based on preliminary experiments and was aimed to encompass the whole response range from no effect on O_2 evolution to total inhibition. A dose-response curve consisted of three replications of six equidistant concentrations on a logarithmic scale, by using a dilution factor of 3.0, plus an untreated control. The dilution factor for bentazone was 4.0 to ensure that the whole response curve was described. The concentration range was from 0.01 to 3 μM for sorgoleone analogues, 0.3 to 10 μM for diuron, and 0.15–150 μM for bentazone.

The ratios of mixtures were chosen from I_{50} values obtained with inhibitors tested alone. By assuming that the Additive Dose Model would apply, the putative $\log(I_{50})$ of mixtures was aimed at being evenly spaced between the two inhibitors when applied alone.[10]

2.4 Dose-response model

The response, O_2 evolution, (U) on concentration of inhibitor, (z) was assumed to be well described by the logistic model[12,13]

$$U_j = C + \frac{D - C}{1 + \exp\{b[\log(z_j) - \log(I_{50})]\}}, \quad (1)$$

where U_j denotes O_2 evolution at the jth dose; D and C denote the upper and lower limits of O_2 evolution at zero and at infinite concentrations of inhibitor. I_{50} denotes the concentration required to reduce O_2 evolution by half between the upper and lower limits, D and C; and b is proportional to the slope of the curve around I_{50}.

The I_{50} is usually considered a 'natural' parameter of the logistic response curve. Any other response level (eg I_{20}, I_{80}, etc.) can, however, conveniently be expressed by a function of the parameters, b and I_{50}, in that

$$I_{50} = \frac{I_x}{\left(\frac{x}{100 - x}\right)^{1/b}} \quad (2)$$

where x is any response level. For example, for I_{20}, x is equal to 20. Consequently, if we estimate the parameters of the response curves, we can analyse the joint action at other response levels than that of I_{50}.

An experiment consists of up to five dose-response curves. As the O_2 evolution was measured consecutively for each dose response curve, we must analyse the response curves separately, because we cannot consider them to have the same variance.[14] The method of stabilizing the variance of response by using a Transform-Both-Sides method on the non-linear regression and the tests for lack of fit of the models have been described elsewhere.[11–16]

2.5 The additive dose model

The assessment of the joint action of mixtures is based upon the Additive Dose Model (ADM).[8,16–19] If Z_1 and Z_2 are concentrations of two inhibitors applied singly, the relative potency between Z_1 and Z_2 is r ($r = Z_1/Z_2$); and $z_1 + z_2$ is a mixture giving the same response, say I_{50}, then ADM is:

$$Z_1 = rZ_2 = z_1 + rz_2 \quad (3)$$

Equation (3) shows that, with ADM, any one mixture can be unambiguously defined by either of the inhibitors applied alone. For a mixture concentration, z_m, consisting of a proportion p of z_1 and (1-p) of z_2, eqn (3) can be rewritten

$$Z_1 = z_m[p + r(1 - p)] \quad (4)$$

Provided ADM holds and Z_1, z_m and p are known, the relative potencies between the herbicides applied singly can be calculated without knowing Z_2.[15] In fact, if ADM can be entertained, we can express one mixture in terms of two other mixtures and thus the activity of each inhibitor can be predicted on the basis of the mixtures themselves.[10]

An extension of ADM, which can predict the shape of the isobole, be it linear (=ADM), convex (the compounds detract from each other's action, antagonism), or concave (the compounds increase each other's action, synergism), was suggested by Vølund[20] and has recently been applied to herbicide research.[11] It requires, however, that the response curves can be fitted simultaneously by a regression model and that all curves are similar in all parameters but that of the relative potency.

3 RESULTS AND DISCUSSION

Summaries of the O_2 evolution experiments are found in Tables 1 and 2 and Figs 2–6. The residual variance of response curves was not always the same within an experimental run, therefore we analysed each response curve separately. All regressions in Tables 1 and 2 had a non-significant test for lack of fit.

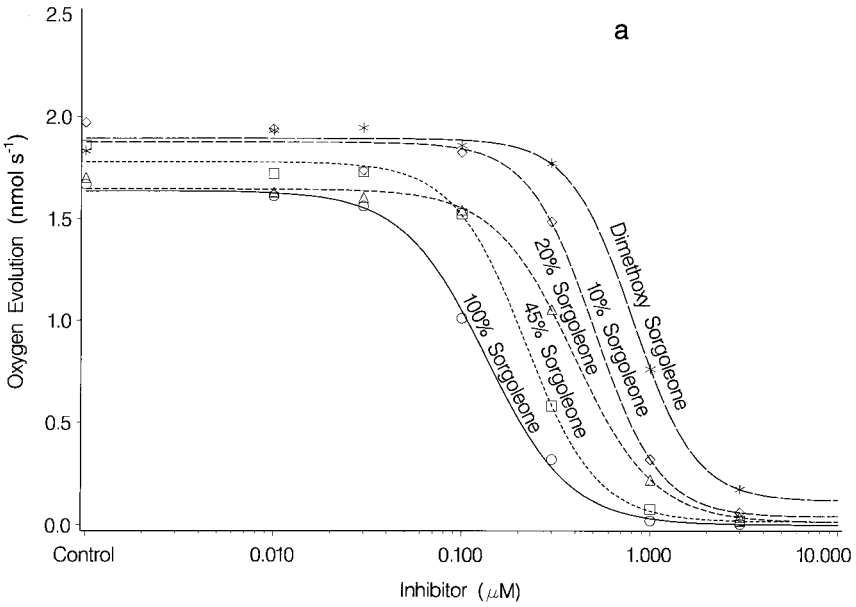
The individually fitted response curves for experiment 1 in Table 1 are displayed in Fig 2(a). The lower and upper limits of the curves diverge somewhat, as apparently do the slopes. Scaling the O_2 evolution measurements by the upper and lower limits, D and C, of the logistic regressions indicated that the curves have, with a single exception, the same slope [Fig 2(b)].

Because the slope of the logistic curve is scale-independent, we can test whether slopes of the curves within an experiment are statistically different. In Table 1, the slope of 2.05 for the 20% sorgoleone mixture was not significantly different from that of 2,5-dimethoxysorgoleone (2.61). In experiment 6, the response curve for the pure 5-ethoxysorgoleone was significantly shallower than those of sorgoleone alone and the mixtures with 33 or 15% sorgoleone, whilst the 5% sorgoleone mixture had an intermediate slope (Fig 3).

By testing the slopes for the pure inhibitors within experiments in Table 1, four out of six experiments had non-significant differences in slope, whilst all five experiments in Table 2 had significantly different slopes.

Dealing with a site-specific assay, we had anticipated *a priori* that the slopes of the response curves would be similar, since these inhibitors have the same site of action. Surprisingly, this was not always the case (Tables 1 and 2, Figs 2 and 3). For the sorgoleone analogues applied alone, most of the slopes were similar, ranging from 1.17 to 2.66, and in only two out of six experiments were they significantly different. It would therefore be reasonable to assume that the slopes of response curves for the sorgoleone analogues were essentially similar. Further experiments supported this view (data not shown). In all instances, the slopes of the synthetic PSII inhibitors were significantly different from those of sorgoleone analogues, 1.51 and 1.64 for diuron and between 0.44 and 0.65 for bentazone.

This consistent difference in slopes was found in numerous experiments where the compounds were assayed together (data not shown). This unexpected result may have significant impact when using ADM. Indeed, if further evidence of different slopes



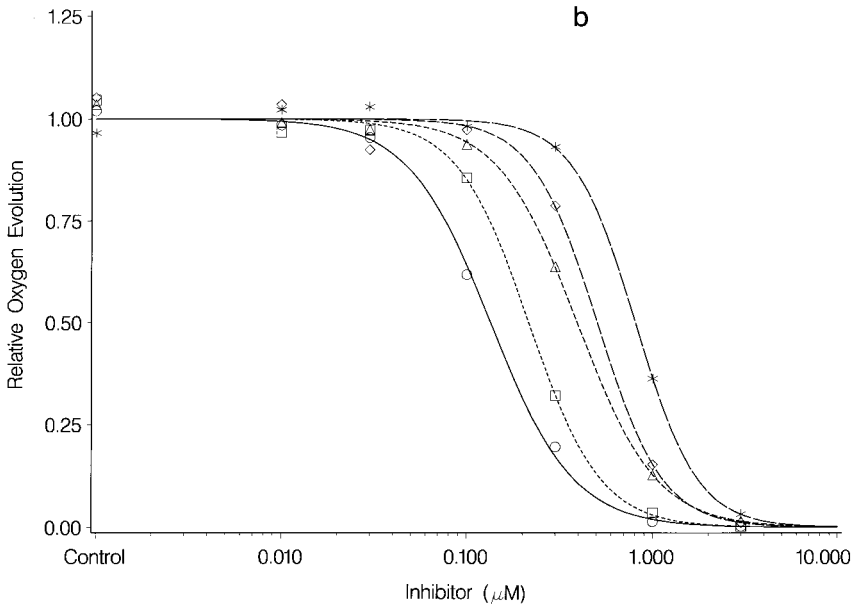


Figure 2. Dose-response curves (experiment 1) (a) on the original scale and (b) after scaling by the upper and lower limits, *D* and *C*.

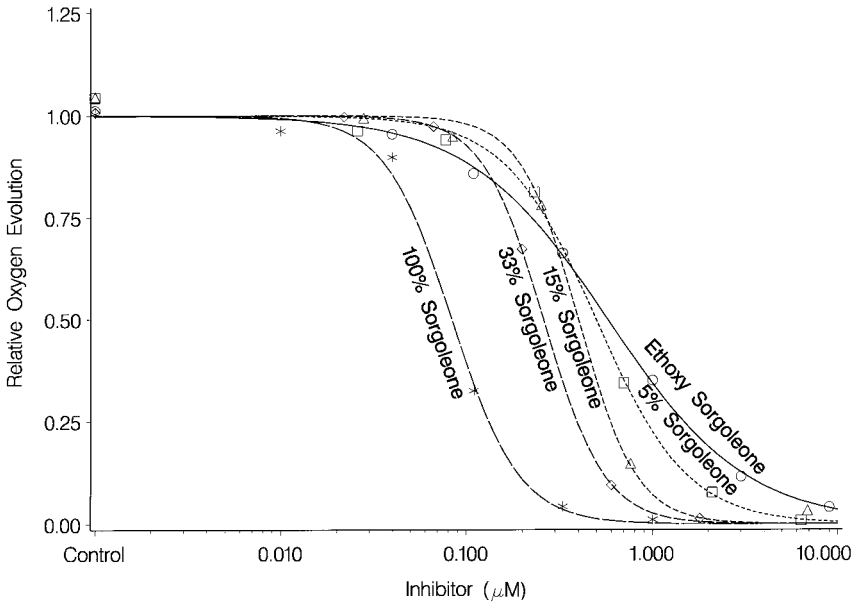


Figure 3. Dose-response curves (experiment 6) after scaling by the upper and lower limits, *D* and *C*.

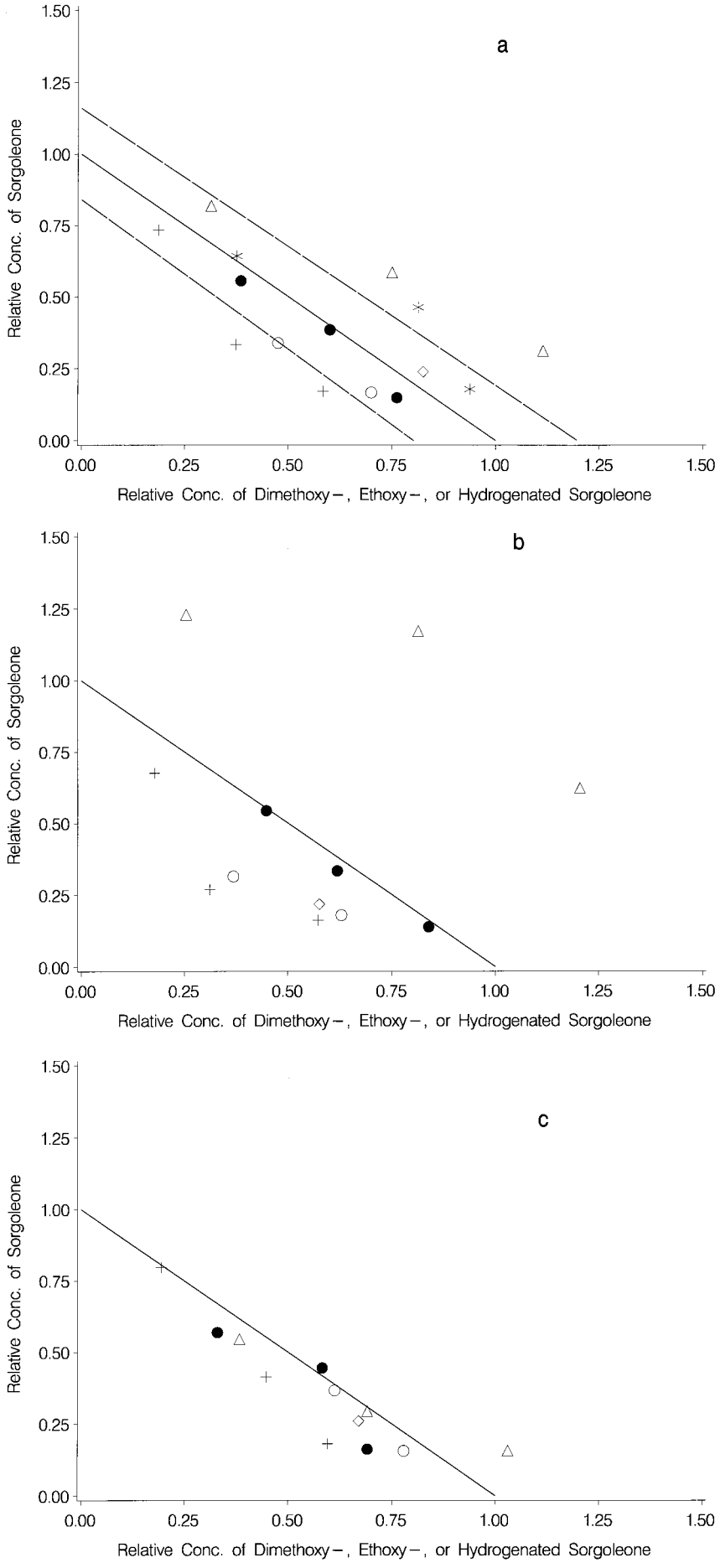


Figure 4. ADM isobole and estimated concentrations of mixtures from experiments in Table 1 on the (a) I_{50}, (b) I_{20} and (c) I_{80} level. The concentrations have been scaled so that the concentration of sorgoleone species applied singly at the chosen *I* level is 1.00. Symbols for experiments, (●) no 1; (+) no 2; (°) no 3; (*) no 4; (◇) no 5 and (△) no 6. Isobole confidence limit at I_{50} from confidence intervals for sorgoleone analogues used singly (experiment 2).

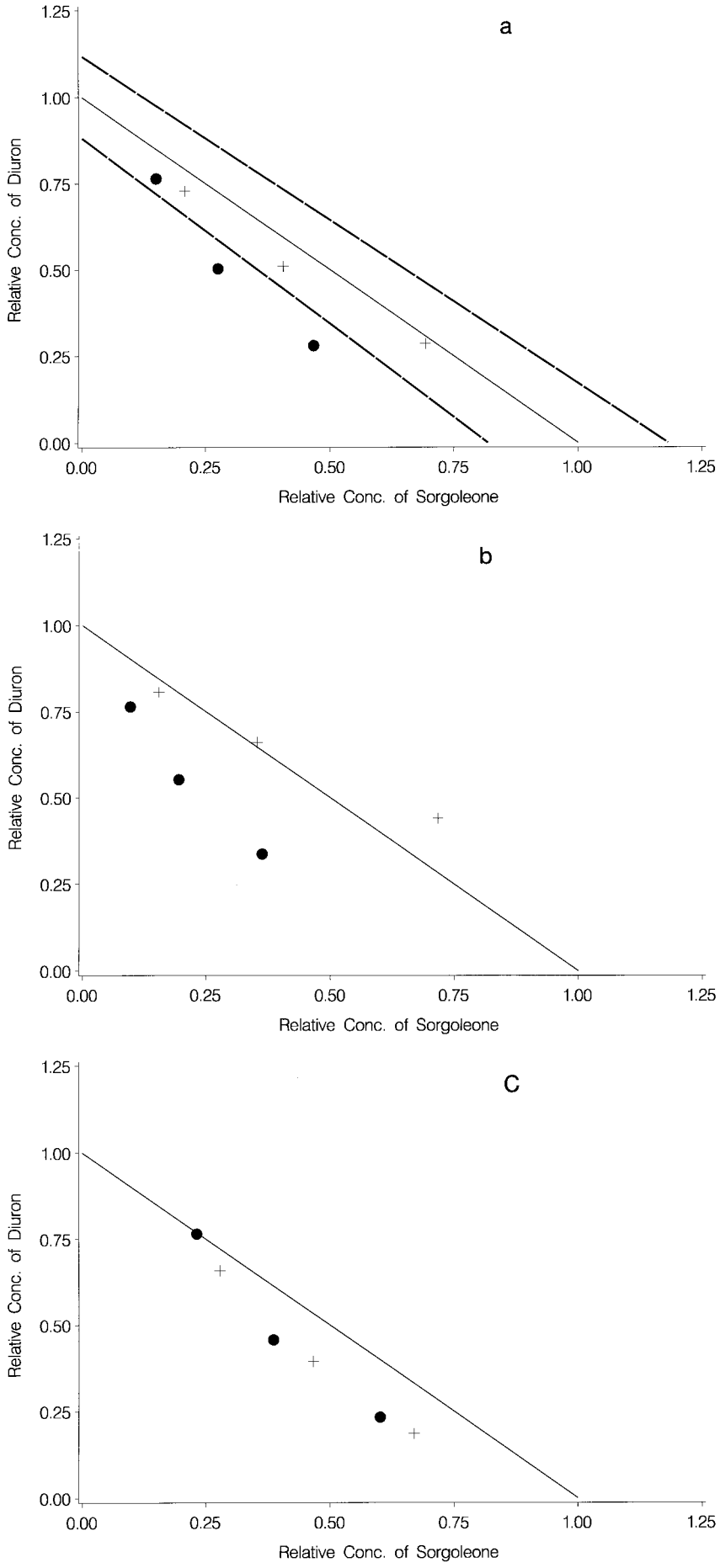


Figure 5. ADM isobole and estimated concentrations of sorgoleone diuron mixtures in Table 2 on (a) I_{50}, (b) I_{20} and (c) I_{80} level. The concentrations have been scaled so that the concentration of the inhibitors applied singly at the chosen I level is 1.00. Symbols for experiments (+) no 1 and (●) no 2; Isobole confidence limit at I_{50} from confidence intervals for sorgoleone and diuron used singly (experiment 2).

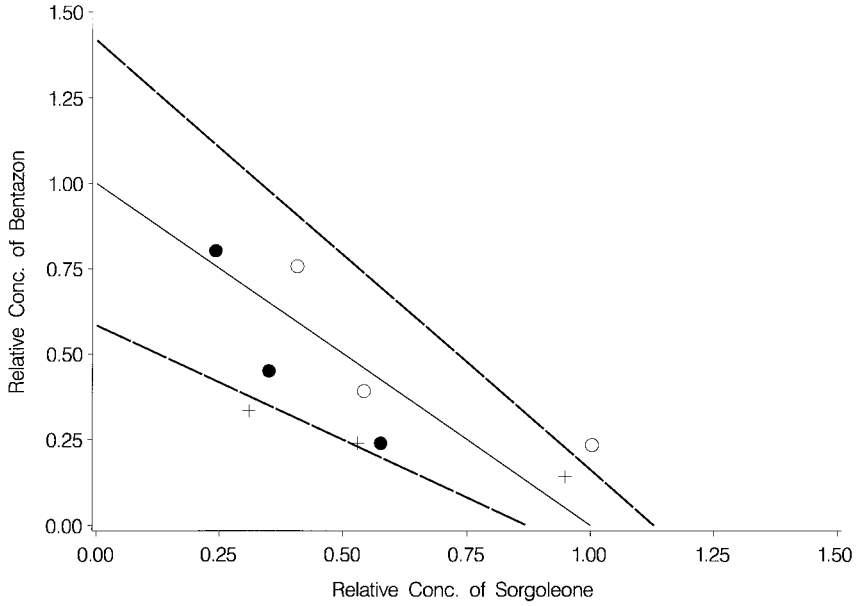


Figure 6. ADM isobole and estimated concentrations of sorgoleone bentazone mixtures in Table 2 on the I_{50} level. The concentrations have been scaled so that the concentration of sorgoleone and bentazone applied singly at I_{50} level is 1.00. Symbols for experiments (●) no 3; (+) no 4 and (°) no 5. Isobole confidence limit from confidence intervals for sorgoleone and bentazone used singly (experiment 3).

in the response curves between the sorgoleone analogues and the synthetic inhibitors is found, then the often-adopted axiom that compounds having the same site of action should have similar slopes must be revised. The difference in the slopes between sorgoleone analogues and synthetic inhibitors may be due to the fact that they interact with different amino acids at the Q_B binding site.

The I_{50} values of sorgoleone applied alone ranged from 0.06 to 0.18 μM (Tables 1 and 2) whilst that of 2,5-dimethoxysorgoleone ranged from 0.17 to 0.81 μM and that of 5-ethoxysorgoleone was 0.58 μM (Table 1). The variation in potency between pure inhibitors within an experiment illustrated how important it is to assay the inhibitors alone and in mixture simultaneously.

In experiment 5 (Table 1), the response curve for hydrogenated sorgoleone (2-hydroxy-5-methoxy-3-pentadecyl-*p*-benzoquinone) alone was missing, but on the basis of the available data, and assuming that the mixtures follow ADM [eqns (3) and (4)], the predicted I_{50} for hydrogenated sorgoleone in this particular experiment would be 0.14 μM, which is not different from its I_{50} measured previously.[21]

The differences in the slope between inhibitors will inevitably influence the distribution of mixtures around the ADM isoboles. When we deal with similar curves, as sometimes can be entertained for whole-plant assays, the interpretation of the distribution of mixtures around the isobole is easy, in that it would be the same whatever the response level.[11,19] When the slopes of the response curves differ, it is likely that the distribution of mixtures relative to the ADM isobole will differ and thus may blur the interpretation (Figs 4–6). The most robust response level which is not too sensitive to the slope of the curve is probably the I_{50} area, but the shallower the slope the more variable the I_{50} value would be (Figs 4a–6a).

Figure 4 shows the distribution of mixtures around the ADM isobole for the sorgoleone analogues in Table 1. The approximate confidence interval of the I_{50} isobole line was calculated according to Gessner.[22] The estimation of mixtures at the I_{20} and I_{80} levels was based on eqn (2). At the I_{50} (Fig 4a), the approximated confidence interval for the isobole (as an example experiment 2 is used), taken from the confidence intervals of the inhibitors applied alone, is indicated. Apart from experiment 6, the distribution around ADM suggests that the joint action of the sorgoleone analogues is additive. Even though one of the mixture response curves (Fig 2) for experiment 1 had a seemingly different slope, it did not drastically change the distribution of the mixtures around the isobole at any response level. Having significantly different slopes, the mixtures from experiment 6 at the I_{20} level showed extreme antagonism. This deviation from ADM at the I_{20} level of response was caused by the previously mentioned rather shallow slope of the response curve for 5-ethoxysorgoleone applied singly and the mixture curve with only 5% sorgoleone (Fig 3).

Table 2 and Figs 5 and 6 give summaries of regression analysis and the distribution of mixtures of sorgoleone with synthetic PSII inhibitors around the ADM isobole. For diuron in Fig 5 at I_{20}, I_{50}, and I_{80}, the distribution of estimated mixtures varied somewhat around the isobole, depending on the response level, partly because the slopes of the diuron curves in Table 2 were significantly lower than those of sorgoleone. Nonetheless, there was no indication of dramatic deviation of the mixtures from the ADM isobole. The distribution around the I_{50} for mixtures of sorgoleone and bentazone (Fig 6) called for the same interpretation as for the mixtures of diuron and sorgoleone, the mixtures acting additively. The differences in slopes between sorgoleone and bentazone

Sorgoleone (%)	D	C	b	I_{50}	
	O_2 Evolution ($\mu mol\, ml^{-1}\, s^{-1}$)			μM	Confidence interval
		With 2-5-dimethoxysorgoleone			
		Experiment 1			
0	1.89 (0.04)	0.12 (0.05)	2.61 (0.64)	0.81	0.70–0.91
10	1.88 (0.04)	0.04 (0.02)	2.50 (0.25)	0.50	0.43–0.57
20	1.65 (0.02)	0.01 (0.01)	2.05 (0.13)	0.39	0.36–0.43
45	1.78 (0.04)	0.02 (0.01)	2.27 (0.22)	0.22	0.19–0.24
100	1.64 (0.06)	—	1.96 (0.16)	0.13	0.11–0.16
		Experiment 2			
0	1.21 (0.03)	0.04 (0.01)	1.83 (0.20)	0.17	0.14–0.20
10	1.45 (0.03)	0.04 (0.01)	1.65 (0.12)	0.14	0.13–0.16
33	1.40 (0.05)	0.05 (0.02)	1.42 (0.16)	0.09	0.07–0.11
60	1.48 (0.04)	0.03 (0.01)	1.70 (0.15)	0.07	0.06–0.09
100	1.24 (0.04)	0.02 (0.01)	1.74 (0.22)	0.08	0.06–0.91
		Experiment 3			
0	1.39 (0.01)	0.01 (0.02)	1.77 (0.11)	0.42	0.38–0.45
25	1.48 (0.02)	0.05 (0.02)	1.61 (0.13)	0.19	0.17–0.21
50	1.47 (0.02)	0.04 (0.01)	1.94 (0.12)	0.14	0.13–0.15
100	1.43 (0.02)	0.04 (0.01)	2.29 (0.16)	0.10	0.09–0.11
		Experiment 4			
0	1.63 (0.04)	0.33 (0.10)	1.18 (0.24)	0.42	0.25–0.59
33	0.92 (0.04)	—	1.09 (0.12)	0.15	0.09–0.20
100	1.58 (0.05)	0.01 (0.01)	1.52 (0.14)	0.06	0.05–0.07
		With hydrogenated sorgoleone (2-hydroxy-5-methoxy-3-pentadecyl-*p*-benzoquinone)			
		Experiment 5			
0				0.14[a]	
25?	1.09 (0.03)	0.06 (0.01)	2.08 (0.27)	0.12	0.10–0.14
50	1.09 (0.04)	0.04 (0.01)	2.64 (0.44)	0.13	0.10–0.15
75	1.12 (0.04)	0.05 (0.01)	2.44 (0.44)	0.10	0.08–0.12
100	1.05 (0.03)	0.08 (0.01)	1.93 (0.21)	0.08	0.07–0.09
		With 5-ethoxysorgoleone			
		Experiment 6			
0	1.26 (0.02)	0.06 (0.02)	1.17 (0.08)	0.58	0.50–0.65
5	1.21 (0.02)	0.13 (0.01)	1.78 (0.17)	0.50	0.44–0.56
15	1.28 (0.03)	0.17 (0.01)	2.81 (0.30)	0.40	0.34–0.46
33	1.25 (0.04)	0.09 (0.01)	2.79 (0.39)	0.27	0.22–0.31
100	1.35 (0.05)	0.04 (0.01)	2.41 (0.31)	0.08	0.07–0.10

Table 1. Summary of regressions with mixtures of sorgoleone analogues (SDs in parentheses)

[a] Calculated on the basis of the ADM isobole ($z_2 = -1.57z_1 + 0.14$), where z_2 is hydrogenated sorgoleone and z_1 is sorgoleone

were greater here than for sorgoleone and diuron. Therefore, the distribution of the mixtures around the isobole would depend even more on the response level. As a result, the mixture ratios were very ill-defined for the I_{20} level because of drastic changes in the potency differences among mixtures and the pure inhibitors. The confidence interval for the isobole clearly shows that, in contrast to the other inhibitors, the variation for bentazone alone was considerable. This is due to the very shallow slope which makes it difficult to estimate a reliable lower limit for the logistic curve.

In conclusion, if the mode of action of sorgoleone analogues is similar to that of synthetic inhibitors of PSII, it would be reasonable to expect that the joint action of sorgoleone analogues, as well as that of mixtures of sorgoleone analogues with synthetic inhibitors, all follow the Additive Dose Model. The unexpected differences in slopes of the curves for the different inhibitors, however, complicate the interpretation. The slopes may indicate that the sorgoleone analogues and the synthetic inhibitors interact differently at the Q_B binding site. Consequently, the joint action of mixtures only applies to the response level considered. If response levels other than I_{50} need to be studied, the choice of mixture ratios should be based on the response level chosen *a priori* rather than on I_{50}.

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Sorgoleone (%)	D	C	b	I_{50}	
	O_2 Evolution ($\mu mol\, ml^{-1}\, s^{-1}$)			μM	Confidence interval
		With diuron			
		Experiment 1			
0	1.81 (0.04)	−0.03 (0.009)	1.51 (0.11)	0.21	0.18–0.24
15	1.20 (0.03)	0.04 (0.01)	1.70 (0.19)	0.18	0.15–0.20
33	1.25 (0.03)	0.02 (0.007)	2.10 (0.17)	0.16	0.13–0.18
60	1.10 (0.02)	0.08 (0.01)	2.85 (0.25)	0.15	0.14–0.17
100	1.23 (0.06)	0.02 0.008	2.66 (0.35)	0.13	0.11–0.16
		Experiment 2			
0	2.17 (0.04)	0.05 (0.02)	1.64 (0.12)	0.20	0.17–0.22
15	2.08 (0.04)	−0.003 (0.01)	1.64 (0.11)	0.18	0.15–0.20
33	1.60 (0.06)	0.04 (0.02)	1.85 (0.27)	0.15	0.12–0.18
60	1.53 (0.03)	0.03 (0.01)	2.10 (0.14)	0.14	0.12–0.15
100	1.33 (0.04)	0.03 (0.01)	3.40 (0.51)	0.18	0.14–0.21
		With bentazone			
		Experiment 3			
0	1.87 (0.06)	−0.02 (0.07)	0.52 (0.06)	1.79	1.04–2.53
4	1.76 (0.09)	0.06 (0.01)	1.10 (0.11)	0.62	0.45–0.8
9	1.59 (0.05)	0.07 (0.02)	1.33 (0.13)	0.47	0.37–0.57
23	1.60 (0.09)	0.10 (0.03)	0.97 (0.40)	0.33	0.24–0.43
100	1.91 (0.06)	0.10 (0.01)	2.52 (0.29)	0.08	0.07–0.09
		Experiment 4			
0	0.95 (0.04)	0.03 fixed	0.44 (0.03)	1.33	0.65–2.00
2	1.04 (0.03)	0.03 (0.01)	1.78 (0.14)	1.09	0.95–1.24
5	1.01 (0.02)	0.05 (0.01)	1.33 (0.09)	0.63	0.54–0.71
14	0.91 (0.02)	0.06 (0.01)	2.27 (0.22)	0.37	0.28–0.46
100	1.01 (0.03)	0.03 (0.01)	2.51 (0.37)	0.09	0.08–0.11
		Experiment 5			
0	1.09 (0.03)	0.10 (0.02)	0.65 (0.07)	1.58	1.00–2.16
2	0.98 (0.03)	0.02 (0.01)	1.48 (0.12)	1.22	1.00–1.45
5	0.96 (0.02)	0.09 (0.01)	2.02 (0.14)	0.65	0.58–0.71
14	0.95 (0.02)	0.12 (0.01)	3.41 (0.91)	0.43	0.38–0.48
100	0.96 (0.03)	0.07 (0.01)	2.40 (0.30)	0.06	0.05–0.07

Table 2. Summary of regressions with mixtures of sorgoleone and synthetic PSII inhibitors (SDs in parentheses)

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